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The impact of divergent breed types and diets on methane emissions, rumen characteristics and performance of finishing beef cattle

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Short title: Methane emissions and performance of beef cattle

Abstract

This study was undertaken to further develop our understanding of the links between breed, diet and the rumen microbial community and determine their effect on production characteristics and methane (CH_4) emissions from beef cattle. The experiment was of a two × two factorial design, comprising two breeds (CHX, crossbred Charolais; LU, purebred Luing) and two diets (concentrate-straw or silage-based). In total, 80 steers were used and balanced for sire within each breed, farm of origin, and BW across diets. The diets (fed as total mixed rations) consisted of (g/kg dry matter (DM)) forage to concentrate ratios of either 500:500 (Mixed) or 79:921 (Concentrate). Steers were adapted to the diets over a four week period and performance and feed efficiency were then measured over a 56 day test period. Directly after the 56 day test, CH_4 and carbon dioxide (CO_2) emissions were measured (six steers / week) over a 13 week period. Compared to LU steers, CHX steers had greater average daily gain (ADG; $P<0.05$) and significantly ($P<0.001$) lower residual feed intake. CHX steers had superior conformation and fatness scores ($P<0.001$) than LU steers. Although steers consumed, on a DM basis, more Concentrate than Mixed diet ($P<0.01$), there were no differences between diets in either ADG or feed efficiency during the 56 day test. At slaughter, however, Concentrate-fed steers were heavier ($P<0.05$) and had greater carcass weights than Mixed-fed steers ($P<0.001$). Breed of steer did not influence CH_4 production, but it was substantially lower when the Concentrate rather than Mixed diet was fed ($P<0.001$). Rumen fluid from Concentrate-fed steers contained greater proportions of propionic acid ($P<0.001$) and lower proportions of acetic acid ($P<0.001$), fewer archaea ($P<0.01$) and protozoa ($P=0.09$) but more *Clostridium* Cluster XIVa ($P<0.01$) and *Bacteroides* plus *Prevotella* ($P<0.001$) than Mixed-fed steers. When the CH_4 to

CO_2 molar ratio was considered as a proxy method for CH_4 production (g/kg DM intake), only weak relationships were found within diets. In conclusion, while feeding Concentrate and Mixed diets produced substantial differences in CH_4 emissions and rumen characteristics, differences in performance were influenced more markedly by breed.

Keywords: beef cattle, concentrate, forage, methane, performance

Implications

The effects of diet and breed on steer performance and methane (CH_4) emissions were measured. Methane emissions on a high concentrate (920 g/kg DM) diet were less (0.68) when compared to a mixed forage / concentrate (500 g/kg DM) diet. Although energy lost as CH_4 was reduced on the high concentrate diet, animal performance and carcass quality did not differ between diets. The CH_4 to CO_2 ratio in expired air did not relate well to daily CH_4 production and may therefore have limited use as a proxy for daily CH_4 production.

Introduction

Ruminant livestock systems are under continued political pressure to reduce their greenhouse gas (GHG) outputs. Worldwide, beef production systems generate 2.9 Mt of CO_2-Equivalent emissions per year and CH_4 emissions accounted for 44% of total GHG emissions (Gerber *et al.*, 2013b). The global human population is expected to exceed 9 billion by 2050, with meat consumption projected to increase by more than 75% compared to 2005 (Alexandratos and Bruinsma, 2012). Achieving

this level of production, whilst reducing the environmental impact of ruminant livestock production, represents a considerable challenge.

Ruminants play a crucial role in food security, being able to convert forages and non-human edible food into products for human consumption through enteric fermentation of cellulosic carbohydrates. However, enteric fermentation is the main source of ruminant emissions, as CH_4 is one end product of the microbial digestion process. Methane formation in the rumen depends both on a supply of hydrogen (H_2) from fermentation of feed by bacteria and protozoa and the subsequent conversion of H_2 and carbon dioxide (CO_2) to CH_4 by methanogenic archaea. Enteric CH_4 emissions also represent a loss of gross energy to the animal (estimated at 6-10%), which could be used by the animal for production (e.g. deposition of lean meat) (Cottle *et al.,* 2011; Gerber *et al.,* 2013a and 2013b). Understanding the mechanisms of methanogenesis and the microorganisms involved is important for devising sustainable mitigation strategies to lower the environmental impact of ruminant livestock production.

Recently Rooke *et al.* (2014) reported that CH_4 emissions were less (0.62 of mixed diet) when a diet containing 900 g concentrates / kg dry matter (DM) (concentrate diet) was fed compared to a diet containing 500 g concentrates /kg DM (mixed diet); further, rumen microbial communities were influenced by the genotype and CH_4 emissions by the sire of cattle (Roehe *et al.,* 2016). In the same study, Wallace *et al.* (2014) demonstrated a positive relationship between the relative abundance of archaea in rumen samples taken at slaughter and the quantities of CH_4 produced by individual animals. Furthermore, Wallace *et al.* (2015) has previously demonstrated the influence of microbial communities on CH_4 emissions, and Roehe *et al.* (2016) the impact of the host genetics on CH_4 emissions. Although accurate

measurements of CH_4 emissions using respiration chambers are required to develop and test the effectiveness of CH_4 mitigation strategies, for genetic selection of cattle producing lower CH_4 emissions, methods capable of screening large numbers of animals are required such as sampling animals at slaughter (Wallace *et al.,* 2014). In the present study, the same nutritional strategy of Rooke *et al.* (2014) was used. The hypotheses addressed were that CH_4 emissions expressed on a live-weight gain or carcass yield basis would be lower on a high concentrate diet and that differences between breeds in CH_4 emissions would be greater when genetically more diverse breeds of cattle (Charolais and Luing) were tested.

Material and methods

This study was conducted at the Beef and Sheep Research Centre, SRUC, UK. The experiment was approved by the Animal Experiment Committee of SRUC and was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986.

Experimental design, animals and diets

The experiment was of a two × two factorial design, comprising two breeds (CHX, crossbred Charolais; LU, purebred Luing) and two diets (concentrate-based or silage-based). The breed types were selected to represent two commercially relevant breeds where CHX cattle represent a beef breed known for fast growth and excellent carcass conformation, whilst the LU breed is a more extensively managed hardy hill and upland breed. Two diets (as total mixed rations) were generated using a diet mixing wagon and consisted of (g/kg DM) forage to concentrate ratios of either 500:500 (Mixed) or 79:921 (Concentrate). The ingredient and chemical composition

of the experimental diets are given in Table 1 and the chemical composition of individual components in Table 2. The DM contents of individual components were determined on duplicate samples twice weekly. Bulked feed samples (four per component) were analysed for DM, ash, crude protein, acid detergent fibre, neutral detergent fibre, acid hydrolysed ether extract (AHEE), starch and neutral cellulase and gammanase digestibility (NCGD) (Ministry of Agriculture Fisheries and Food, 1992) and gross energy by adiabatic bomb calorimetry. ME values (Thomas, 2004), were either estimated from near infra red spectroscopy (silage and whole crop barley silage), from NCGD and AHEE (barley and wheat distillers dark grains) or from tabulated values for feed composition (straw and molasses).

In total, 80 steers were used (n=40 per diet) and each diet was allocated to two pens (four pens in total; 20 steers per pen). Pens were balanced for sire within each breed, farm of origin and BW and were balanced across diets at the start of the experiment. Fresh water was provided *ad libitum* using a water trough, and diets were offered at approximately 1.05 times average daily intake to all steers using 32 electronic feeders (HOKO, Insentec, Marknesse, The Netherlands). All steers were bedded on wood fibre and sawdust to ensure that consumption of bedding did not contribute to nutrient intake. All steers were fed the Mixed diet before being adapted to diets. Steers allocated the Concentrate diet, were adapted to the full concentrate inclusion over four weeks. Forage to concentrate ratios were increased at weekly intervals such that ratios of 38:62, 25:75, 13:87 and 8:92 were offered during adaptation. During this period, steers were trained to use the electronic feed intake recording equipment.

56-day performance test

After adaptation to the experimental diets, performance and feed efficiency were characterised for all steers over a 56 day test period (day 0 to day 56). Animals were maintained under controlled conditions, where group sizes within the pen remained constant. Individual DM intakes (DMI, kg/day) were recorded for each animal using the electronic feeding equipment and BW measured weekly before fresh feed was offered using a calibrated weigh scale. Ultrasonic fat depth was obtained at the 12th/13th rib at the start (FD0) and end (FD1) of the 56 day test using industry-standard equipment (Aloka 500, BCF Technology LTD, Scotland, UK). Images were analysed using Matrox Inspector 8 software (Matrox Video and Imaging Technology Europe Ltd., Middlesex, UK).

Emissions measurement in respiration chambers

Directly after the 56 day performance test, 72 steers were allocated to six respiration chambers over a 12 week period using a randomised block design (six chambers times four weeks) which was repeated three times. Within each block, each treatment of the two × two factorial (breed × diet) experimental design was replicated once in each respiration chamber. Steers were allocated to blocks to minimise variation in BW (mean BW (kg) 617, SEM 6.6) on entry to the respiration chambers. The steers remained in the respiration chambers for three days, during which time they were fed once daily and had *ad-libitum* access to feed. Data for DMI during the three day chamber measurement period were averaged per animal. One chamber malfunctioned during weeks 6 to 10, which resulted in the requirement for an additional week of chamber measurement; thus measurements were made from 73 steers.

Full details of the six indirect open-circuit respiration chambers (No Pollution Industrial Systems Ltd., Edinburgh, UK) and their operation are given in Rooke *et al.* (2014) and Troy *et al.* (2015). In addition to CH_4, CO_2 concentrations were also measured by infrared absorption spectroscopy (MGA3000, Analytical Development Co. Ltd., Hoddesdon, UK) after calibration with a gas mixture of known composition. Prior to the beginning of the experiment, gas recoveries were measured by releasing CO_2 at a constant rate into each chamber. To accustom the steers to the chamber environment, six days prior to chamber measurements groups of steers were moved to the building in which chambers were located and loose-housed in single pens (4 × 3 m) of identical design to pens within the chambers. After six days, the steers were then moved to the chambers and remained there for 72 h, with CH_4 and CO_2 measurements recorded in the final 48 h used in further analysis. Steers were fed (at approximately 1.05 times average daily intake) once daily and weight of feed within the bins recorded at 10 sec intervals using load cells. Front doors of chambers were briefly opened at about 08.00 h daily to remove feed bins and again to replace bins with fresh feed at approximately 09.00 h. The pens were cleaned daily between 08.00 and 09.00 h. The exact times when doors were opened were recorded.

Rumen sampling, volatile fatty acid and microbial analyses

Immediately after the steers (within two hours) left the respiration chambers, samples of rumen fluid were obtained (one per animal) by inserting a tube (16 × 2700 mm Equivet Stomach Tube, Jørgen Kruuse A/S, Langeskov, Denmark) nasally and aspirating manually. Approximately 50 mL fluid were strained through two layers of muslin and samples prepared for VFA analysis and DNA extraction prior to storage at -20 °C as previously described (Rooke *et al.,* 2014). Similarly, DNA extraction was

carried out using a method based on repeated bead beating plus column filtration and qPCR methodology to quantify relative abundance of microbial groups in rumen samples (Rooke *et al.,* 2014).

Pre-slaughter measurements and carcass quality

Other than for measurements of CH_4 emissions within the respiration chamber facility, steers remained within the same pens from the end of the 56 day test to slaughter. All steers remained on the same diet throughout the experiment. On the day before slaughter, ultrasonic fat depth (FD2) at the 12th/13th rib was measured in all steers as described above. Steers were slaughtered in five batches of 6, 21, 18, 15 and 19 steers on days 71, 92, 113, 134 and 155, respectively. Steers were selected for slaughter based on BW and visual assessment of fatness. Steers had access to feed until they left the premises. The steers were transported (approximately 1 h) to a commercial abattoir and slaughtered within 2 h of arrival. Cattle were stunned using a captive bolt, exsanguinated and subject to low voltage electrical stimulation. Following hide removal, carcasses were split in half down the mid-line and dressed to UK specification (see Meat and Livestock Commercial Services Limited beef authentication manual, www. mlcsl.co.uk, for full description). EUROP conformation and fat classifications (Fisher, 2007), based on the UK scale, were allocated to all carcasses through visual assessment using a trained assessor.

Video Image Analysis (VIA) was used to estimate EUROP classifications (conformation and fat), total lean (kg) and total fat (kg) content of the whole carcass. The VIA systems in use in the EU are automatic machines that perform carcass evaluation based on images of the half carcass. The VBS 2000 system used in this study (E+V technology GmbH, Oranienburg, Germany) has been approved by the

Department for Environment, Food and Rural Affairs (Defra) for use in the UK since 2010. The system operated at the end of the slaughter line after all necessary dressing and trimming had been completed. A pneumatically operated cradle presented the left half side of each carcass for imaging. The VIA camera took two images of the half carcass, a 2-dimensional image and a pseudo 3-dimensional image using structured light (Craigie *et al.,* 2012). The VBS 2000 required information on the category of the carcass (i.e., steer) and hot carcass weight (kg) and, by combining this information with data automatically captured by the VIA system (i.e., carcass dimensions, angles, areas, colour), predicted EUROP classification and total lean and fat content of the whole carcass.

Calculations and statistical analysis

Data from two steers during the 56 day test period and one steer at slaughter were unavailable as the animals were removed from the trial for health reasons unconnected to the diets imposed. Growth was modelled by linear regression of BW against test date, to obtain ADG, mid-test BW (mid-BW) and mid-test metabolic BW (mid-MBW, $BW^{0.75}$). Mean DMI over the 56 day period was expressed as kg/day or as a proportion of mid-BW and mid-MBW. Feed conversion ratio (FCR) was calculated as average DMI per day (kg/d) divided by ADG. Residual feed intake (RFI) was calculated as deviation of actual DMI (kg/d) from DMI predicted based on linear regression of actual DMI on ADG, mid-MBW and FD1 (Basarab *et al.,* 2003). Cold carcass weight (CCW) was calculated as a percentage of slaughter BW (SBW) to determine killing out percentage (KO). To allow for statistical comparison, the EUROP carcass classification values were expressed on the equivalent 15 point scale (Kempster *et al.,* 1986). Statistical analyses of performance and carcass data

were conducted using the mixed procedure of SAS software with the fixed effects of breed and diet, and the random effect of pen (and slaughter batch for carcass traits). In addition, in the analysis of FD1 and FD2 the deviation from the breed mean of FD0 was included as a covariable. The interaction effects of breed × diet were included in the model when these effects proved significant (P<0.05).

The respiration chamber measurements from three steers were discarded as the DMI decreased substantially (> 30%) whilst being housed in the respiration chamber, leaving data from a total of 70 individual steers. Rumen fluid samples were not obtained for two steers and therefore 68 individual animal observations were available. Data were analysed using SAS software using linear mixed models. The fixed effects were breed and diet, while the random effects were week and chamber. The effect of the breed × diet interaction was also included in the model when this proved significant (P<0.05).

Data are reported as means with their SEM unless indicated otherwise. Differences between means were tested using a least square means comparison test. Probability values were deemed significant where P<0.05 and indicated a tendency when probability values were between P=0.05 and P=0.1. The numbers of steers in treatments are given in each Table for clarity.

Results

Performance test

Although there were no differences in age at the start of the trial, CHX steers were significantly (P<0.001) heavier than LU steers (Table 3). However, there were no differences between breeds in daily DMI and therefore on a BW basis, LU steers consumed more DM (g/kg BW or g/kg$^{0.75}$, P<0.001) than CHX steers. Compared to

LU steers, CHX steers had greater ADG (P<0.05) throughout the performance trial and lower FD1 (P<0.01) at the end of the trial. CHX steers were more efficient than LU steers as measured by numerically lower FCR and significantly (P<0.001) lower RFI than LU steers.

Although steers consumed more of the Concentrate than Mixed diet (P<0.01), there were no differences between diets in either ADG or feed efficiency (expressed as either FCR or RFI). Fat depth (FD1) tended to be lower (P=0.06) on the Concentrate than Mixed diet.

Carcass traits

CHX steers were superior to LU steers for most carcass traits recorded (Table 4). Thus, CHX were heavier at slaughter with greater KO resulting in greater CCW (all P<0.001). Regardless of measurement method, CHX steers had superior conformation and fatness scores (P<0.001) which were reflected in greater carcass meat and lower carcass fat yields (predicted by VIA).

Concentrate-fed steers were heavier at slaughter (P<0.05) and had greater CCW than Mixed-fed steers (P<0.001). Although there were no differences in carcass scores when visually assessed, the VIA system predicted superior conformation scores (P<0.05) and meat yields (P<0.01) for Concentrate-fed steers.

Methane and carbon dioxide production

Breed of steer did not influence either CH_4 or CO_2 production. Methane production (Table 5), whether expressed as g/day, g/kg DMI or kJ/MJ GE intake, was substantially lower when the Concentrate rather than the Mixed diet was fed

298 (P<0.001). There were no differences between diets in total daily CO_2 production but
299 CO_2 production expressed as g/kg DMI was greater when the Mixed diet was fed.
300 The ratio of CH_4 to CO_2 production (mole/mole) was greater on the Mixed than
301 Concentrate diet (P<0.001). Although, there was a strong linear relationship between
302 CH_4 production (g/kg DMI) and CH_4 to CO_2 molar ratio (P<0.001) when all animals
303 were considered, this was largely due to between–diet differences as within diets, the
304 relationships were much weaker (Fig. 1). However, and irrespective of whether data
305 from all animals were considered together or within diets, essentially most of the
306 variation in CH_4 (g/kg DMI) was explained when both CH_4 to CO_2 ratio and CO_2
307 production (g/kg DMI) were included in models.
308
309 Overall: CH_4 (g/kgDMI) = 159 (16.3) CH_4 to CO_2 molar ratio + 0.0099 (0.00135) CO_2
310 (g/kg DMI); r^2 0.74, P<0.001.
311
312 *Rumen fluid VFA and microbial populations*
313 Rumen fluid from Concentrate-fed steers (Table 6) contained greater proportions of
314 propionic and valeric (both P<0.001) acids but lower proportions of acetic (P<0.001)
315 and butyric (P<0.01) acids than Mixed-fed steers. There were no differences in VFA
316 between breeds. Breed did not influence rumen microbial populations (Table 6).
317 Rumen fluid from Concentrate-fed steers had a lower abundance of archaea
318 (P<0.01) and protozoa (P=0.09) but more bacteria (P<0.001). There were no
319 differences between diets in abundance of *Clostridium* Cluster IV in rumen fluid, but
320 rumen fluid from Concentrate-fed steers contained more *Clostridium* Cluster XIVa
321 and *Bacteroides* plus *Prevotella* than Mixed-fed steers. When the relationship
322 between CH_4 emissions (g/kg DMI) and archaea populations (expressed as ratio of

323 archaea to total bacteria, Wallace *et al.,* 2014) was explored the relationship was
324 significant (*P*<0.001, Fig. 2) but when the Mixed and Concentrate diets were
325 considered individually the relationships were weaker and only significant (*P*<0.05)
326 for the Concentrate diet.
327
328 **Discussion**
329 *Performance*
330 *Diets.* There were few differences in performance traits between the Mixed (500 g
331 concentrate DM / kg total DM) and Concentrate diets in the present study. Feed
332 intake was significantly and ADG numerically greater for the Concentrate than Mixed
333 diet but neither FCR nor RFI differed between diets. Since there were also few
334 differences in carcass composition, after differences in slaughter weight were
335 accounted for, there was little evidence for any underlying differences between diets
336 in the energy content of deposited tissue. These results are similar to the study of
337 Duthie *et al.* (2016) who used the same breeds and similar diets and experimental
338 protocols to the present study. Thus, FCR did not differ between diets and there was
339 little evidence of differences in carcass composition particularly fat content in either
340 study and therefore, there was no advantage to the Concentrate diet in animal
341 performance either in BW, CCW or energetic terms. This lack of difference between
342 diets is in contrast to the expectation from the literature. For example, Lovett *et al.*
343 (2003) reported that heifers offered a concentrate diet (900 g concentrate / kg DM)
344 consumed similar DMI but grew faster (1.1 *v.* 0.8 kg/d) and had superior FCR (8.5 *v.*
345 11.4 kg DMI/ kg ADG) than heifers fed a 600 g concentrate / kg DM diet. The
346 predicted efficiencies of utilisation of metabolisable energy for growth (AFRC 1993;
347 0.50 and 0.54 for Mixed and Concentrate diets) would suggest that the Concentrate

diet could support superior performance and the higher molar proportion of propionic acid on the Concentrate diet would have supplied more precursors for gluconeogenesis and lean tissue deposition. A likely explanation for the lack of difference between the two diets is that the numerically greater ADG for steers fed the Concentrate diet were the maximum ADG possible.

Breeds. The differences in performance between CHX and LU in the present study were similar to Duthie *et al.* (2016). That is, the CHX steers had greater daily ADG and superior FCR. The differences between breeds in slaughter characteristics were also similar between studies; CHX had greater carcass weights and superior EUROP conformation (visually assessed or predicted from VIA) and lower fat depth. In the present study, the quantitative differences between the breeds in performance were lower. In particular, in Duthie *et al.* (2016) LU steers had greater DMI than CHX, but there were no differences in the present study. The reason for this difference is likely that in Duthie *et al.* (2016), steers entered the performance study at the same BW but LU steers were approximately 30 days older and thus nearer maturity especially since LU steers would reach maturity at a younger age than CHX. In this context, if LU are classified as a medium maturing cattle type compared to the CHX, a late maturing type, then from AFRC (1993) the energy value of gain would be 22.2 and 23.3 MJ/kg ADG for CHX and LU respectively. Using these values, the net energy requirements for the observed ADG of 36.8 and 36.6 MJ net energy / day for CHX and LU respectively are little different. Thus in terms of energy efficiency there is little difference between the breeds.

Methane emissions

373 *Diets.* In an experiment of similar design to that reported here (Rooke *et al.,* 2014)
374 but using different breeds of cattle, mean CH_4 emissions (g/kg DMI) were similar
375 (present experiment *v. Rooke et al.,* 2014; Concentrate, 13.9 *v.* 13.6; Mixed, 20.4 *v.*
376 21.8). This difference between diets was consistent with both the literature (Hristov *et*
377 *al.,* 2013) and the observed changes in VFA proportions: increased molar proportions
378 of propionate (hydrogen consuming) and decrease proportions of acetate (hydrogen
379 producing) on the Concentrate diet. Based on many studies, equations to predict CH_4
380 yield which include the proportion of concentrate in the diet have been developed.
381 The equation of Sauvant and Giger-Reverdin (2009) predicted CH_4 yields (expressed
382 as kJ CH_4 / MJ total GE) of 48 and 79 kJ CH_4 /MJ GE intake for the Concentrate and
383 Mixed diets respectively compared to observed means of 42 and 60. The more
384 recent equation for non-lactating cattle developed by Hristov *et al.* (2013) produced
385 values of 59 and 65 kJ CH_4 / MJ GE intake. Both equations thus over-predicted CH_4
386 produced from the Concentrate diet. This may be because of under-representation or
387 absence of high concentrate diets from the prediction data sets. Rooke *et al.* (2014)
388 noted that the value of 39 kJ CH_4 / MJ GE for the Concentrate was higher than
389 values observed for North American feedlot diets (20 – 30 kJ MJ CH_4 / MJ GE) based
390 on maize grain and that this was due to the greater cell wall concentration in barley
391 grain (Beauchemin *et al.,* 2005; Doreau *et al.,* 2011). For the Mixed diet, the value
392 predicted by Hristov *et al.* (2013) was in closer agreement with the observed value
393 than that from Sauvant and Giger-Reverdin (2009) likely because the Hristov *et al.*
394 (2013) equation included terms for NDF and ether extract which more accurately
395 described the nutrient composition of the diet.

396 Breed had no overall effect on CH_4 yield in the present experiment. This was
397 in agreement with our own (Rooke *et al.,* 2014; Duthie *et al.,* 2015; Troy *et al.,* 2015)

398 and other previous studies (Boadi and Wittenberg 2002; Fraser *et al.,* 2014;
399 Richmond *et al.,* 2015) using different breeds. However, Hristov *et al.* (2013) have
400 argued that emissions intensity (CH_4 produced per unit animal product) most
401 accurately represented the potential of a mitigation strategy. Since detailed animal
402 performance records and CH_4 emissions were measured in this experiment, it was
403 appropriate to estimate emissions intensities for the diets fed. In so-doing the
404 limitations imposed by recording animal performance, CH_4 emissions and carcass
405 characteristics consecutively should be noted. As an example, feed intakes
406 expressed as a proportion of BW were greater during the performance trial than the
407 CH_4 measurement period and therefore CH_4 emissions (g/kg DMI) during the
408 performance measurement would likely have been less than those measured later
409 (e.g. Sauvant and Giger-Reverdin 2009). Table 7 shows that whilst the difference
410 between diets within breed remained relatively independent of the method of
411 measurement, the effect of breed was substantial particularly when CH_4 emissions
412 were based on carcass and estimated meat weights with the LU cattle fed the Mixed
413 diet producing nearly twice the amount of CH_4 on a carcass meat basis than CHX
414 cattle fed the Concentrate diet.
415
416 *Rumen microbiota.* In Rooke *et al.* (2014), there was a significant relationship
417 between archaea populations (ratio of archaea to total bacteria) and CH_4 emissions
418 (Wallace *et al.,* 2014) and there were also differences in rumen microbiota between
419 breeds (Rooke *et al.,* 2014). In the present study, there was a similar relationship
420 between CH_4 emissions and archaeal populations (Fig. 2) to Wallace *et al.* (2014)
421 where the relationship was positive and significant for the Concentrate but not the
422 Mixed diet, suggesting that the archaea populations and CH_4 emissions were limited

by available hydrogen on the Concentrate diet (Janssen, 2010). However, in contrast to Rooke *et al.* (2014) there were no differences in rumen microbiota or CH_4 emissions between breeds of cattle. This was despite the fact that the breeds used in the present experiment (CHX and LU) were more genetically divergent than the genotypes used by Rooke *et al.* (2014; Limousin x Aberdeen Angus and Aberdeen Angus x Limousin). A possible explanation for this difference may be the source of the cattle used. Whereas the steers used by Rooke *et al.* (2014) were raised on the farm in which the experiment was carried out, in the present experiment, steers were obtained from nine different farms. It is thus possible that the different farm environments the cattle used in the present experiment were derived from had a greater effect on rumen microbiota than differences between breeds.

Methane and carbon dioxide emissions

Quantifying CH_4 emissions using respiration chambers is a costly and relatively low throughput procedure and there is therefore considerable interest in establishing proxy procedures which are low cost, more rapid and more applicable to the normal farm environment. A possible option within dairy systems is the measurement of CH_4 and CO_2 concurrently from sampling points for example in the dairy parlour (Lassen *et al.,* 2012; Bell *et al.,* 2014b). Both the above studies concluded that the CH_4 to CO_2 phenotype was repeatable. It was proposed by Madsen *et al.* (2010) that by calculating heat production by the animal and converting heat production to CO_2 production, CH_4 to CO_2 ratios could be converted to daily CH_4 emissions. However Bell *et al.* (2014b) found only a poor relationship between average CO_2 production estimated according to Madsen *et al.* (2010) and measured CO_2 concentrations. Factors proposed to explain this lack of agreement by Bell *et al.* (2014b) were animal

448 to animal variation including differences in diurnal pattern of CH_4 to CO_2 ratio, feed
449 intake and fasting heat production itself. This is confirmed in the present study where
450 measurements were made over a 48 h period thus excluding short-term changes in
451 breath CH_4 and CO_2 concentration. Further, since all animals were gaining weight,
452 CO_2 derived from body tissue mobilisation would not have influenced the results. The
453 diets fed influenced CO_2 production and therefore CH_4 to CO_2 ratio with CO_2
454 production (g/kg DMI) being greater for the Mixed diet as expected from differences
455 in VFA pattern. More importantly and particularly within diets, the correlation between
456 CH_4 production (g/kg DMI) and CH_4 to CO_2 ratio was poor (Fig. 1) but variation in
457 CO_2 production in conjunction with CH_4 to CO_2 ratio explained most of the variation in
458 CH_4 production. Thus although the phenotype of CH_4 to CO_2 ratio may be
459 repeatable, the present experiment suggests that it may not relate well to daily CH_4
460 production because of animal to animal variation in extent of digestion, efficiency of
461 utilisation of absorbed nutrients and tissue CO_2 turnover.
462
463 **Conclusions**
464 This large scale, integrative study reported animal performance including carcass
465 characteristics together with measurement of CH_4 emissions and characterised
466 rumen VFA and microbial abundance. In agreement with previous studies (Rooke *et*
467 *al.,* 2014; Duthie *et al.,* 2016) CH_4 emissions were less (0.68 of mixed diet) when a
468 high concentrate diet was fed compared to a mixed forage:concentrate diet.
469 However, although energy lost as CH_4 was reduced by 18 KJ/MJ gross energy
470 intake, there were no differences in animal performance or carcass characteristics
471 between the diets fed. Although breed of steer had no effect on CH_4 emissions, ADG
472 was less and feed conversion efficiency was poorer for LU compared to CHX steers.

Assessment of the CH_4 to CO_2 ratio as a proxy measurement for CH_4 emissions made using respiration chambers, suggests that the ratio may not relate well to daily CH_4 production because of animal to animal variation in digestion and utilisation of feed.

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621 **Table 1** *Ingredient composition and calculated chemical composition of experimental diets*

Diet	Mixed	Concentrate
Ingredient composition, g/kg DM[1]		
Grass silage	215	-
Whole crop barley silage	285	-
Barley straw	-	79
Barley	388	713
Wheat Distillers Dark Grains	103	175
Molasses	-	23
Minerals[2]	9	10
Chemical composition, g/kg DM[3]		
Dry matter (g/kg)	437	862
CP	138	135
ADF	207	112
NDF	337	248
AHEE	39	47
Starch	284	415
Ash	53	32
ME (MJ/kg DM)	12.0	12.8
GE (MJ/kg DM)	19.2	18.6

622 [1]Ingredient composition is the mean of the daily diets received by the animals across the
623 experiment.
624 [2]Contained (mg/kg): Fe, 6036; Mn, 2200; Zn, 2600; Iodine, 200; Co, 90; Cu, 2500; Se 30;
625 (µg/kg): vitamin E, 2000; vitamin B12, 1000; vitamin A, 151515; vitamin D, 2500
626 [3]Chemical composition is the mean of 4 analyses per diet, apart from DM which is the mean
627 of 44 analyses.
628 CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; AHEE, acid
629 hydrolysed ether extract; ME, metabolisable energy; GE, gross energy.
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633 **Table 2** *Chemical composition of feeding stuffs (g/kg DM*)

	Grass silage	WCBS	Straw	Barley	WDG	Molasses
DM (g/kg)	288	298	830	862	851	786
CP	149	111	16	106	321	89
ADF	337	336	547	60	149	0
NDF	393	535	867	169	339	0
Starch	6.0	199.8	16.0	574.3	26.4	0.0
AHEE	37	17	14	33	126	0
Ash	91	66	37	22	58	134
NCGD (% DM)			45	89	78	
ME (MJ /kg DM)	11.9	9.9	6.3	13.3	14.1	12.7
GE (MJ /kg DM)	20.6	19.2	18.1	18.2	22.1	15.5
pH	4.2	4.3				

634 WCBS, whole crop barley silage; WDG, Wheat Distillers Dark Grains; DM, dry matter; CP,
635 crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; AHEE, acid
636 hydrolysed ether extract; NCGD, neutral cellulase and gammanase digestibility; ME,
637 metabolisable energy; GE, gross energy
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Table 3 *Effect of breed (B), diet (D) on growth, feed intake and feed efficiency of Charolais-sired (CHX) and purebred Luing (LU) steers fed either a high concentrate (Concentrate) or mixed forage:concentrate (Mixed) diet during a 56-day performance trial*

Diet	Mixed		Concentrate			Significance		
Breed	CHX	LU	CHX	LU	SEM	B	D	B × D
n of steers	19	19	21	19				
AgeST (days)	394	393	391	391	6.8			
Mid-test BW (kg)	540	476	560	477	13.3	***		
Mid-test MBW ($kg^{0.75}$)	112	102	115	102	2.1	***		
ADG (kg/day)	1.59	1.49	1.73	1.63	0.228	*		
DMI (kg/day)	10.61	10.67	11.73	11.15	0.256		**	
DMI / BW (g/kg)	19.66	22.58	20.95	23.51	1.067	***		
DMI / MBW ($g/kg^{0.75}$)	94.67	105.08	101.87	109.48	4.212	***		
FCR (kg DMI/ kg ADG)	6.74	7.26	6.84	6.97	0.210			
RFI (kg)	-0.643	0.091	0.148	0.427	0.4833	***		†
FD1 (mm)[1]	6.60	7.74	5.98	7.05	0.341	**	†	

AgeST, Age at start of test; MBW, mid-test metabolic BW; ADG, average daily gain; DMI, dry matter intake; FCR, feed conversion ratio; RFI, residual feed intake; FD1, fat depth at the 12/13th[t] rib at the end of the 56 d test; B×D, breed × diet

[1]Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable

***, P<0.001; **, P<0.01; *, P<0.05; †, P<0.1.

649 **Table 4** *Effect of breed (B) and, diet (D) and their interaction on carcass traits of Charolais-sired (CHX) and purebred Luing (LU) steers fed*
650 *either mixed forage-concentrate (Mixed) or high concentrate-based (Concentrate) diets*

Diet	Mixed		Concentrate			Significance		
Breed	CHX	LU	CHX	LU	SEM	B	D	B × D
n of steers	19	20	21	19				
FD2 (mm)[1]	6.92	9.50	7.57	10.4	0.42	***	†	
CCW (kg)	378	305	401	312	7.6	***	***	†
KO (%)	57.3	51.9	57.9	52.3	2.11	***		
SBW (kg)	661	588	694	597	9.3	***	*	
CONF	9.6	7.7	9.6	7.8	0.51	***		
FAT	8.6	10.6	9.3	10.5	0.64	***		†
CONF (VIA)	10.3	7.6	10.8	8.0	0.23	***	*	
FAT (VIA)	6.5	9.3	6.9	8.7	0.75	***		†
TOTAL FAT (kg)	28.03	36.18	34.14	33.75	3.771	*		*
TOTAL MEAT (kg)	270.2	204.7	283.5	214.8	8.95	***	**	

651 FD2, pre-slaughter fat depth at the 12/13th rib; CCW, cold carcass weight; KO, killing out %; SBW, slaughter BW; CONF, EUROP conformation
652 (15 pt scale) assigned by visual assessor; FAT, EUROP fatness (15pt scale) assigned by visual assessor; CONF (VIA), conformation grade
653 (15pt scale) assigned by VIA; FAT (VIA), fatness grade (15pt scale) assigned by VIA; TOTAL FAT; total fat content predicted by VIA; TOTAL
654 MEAT, total meat content predicted by VIA.
655 [1]Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable
656 ***, P<0.001; **, P<0.01; *, P<0.05; †, P<0.1.

Table 5 *Dry matter intakes and methane production from Charolais-sired (CHX) and purebred Luing (LU) steers fed either a high concentrate (Concentrate) or mixed forage:concentrate (Mixed) diets*

Diet (D)	Mixed		Concentrate			Significance		
Breed (B)	CHX	LU	CHX	LU	SEM	B	D	B x D
No of steers	17	19	18	16				
DMI								
kg/day	9.0	9.0	11.0	9.9	0.49		***	†
g/kg BW	14.2	15.8	16.2	16.9	0.78	*	**	
Methane								
g/day	193	184	144	150	11.0		***	
g/kg DMI	20.2	20.7	13.2	14.7	0.64		***	
kJ/MJ GEI	59.1	60.6	39.4	43.6	1.88		***	
Carbon dioxide								
g/day	7468	7034	7685	7376	548.5			
g/kg DMI	788	795	710	730	62.2		*	
Molar ratio								
CH_4:CO_2	0.071	0.072	0.052	0.056	0.004		***	

DMI, dry matter intake; GEI, gross energy intake; CH_4, methane; CO_2, carbon dioxide

***, P<0.001; **, P<0.01; *, P<0.05; †, P<0.1.

664 **Table 6** *Volatile fatty acid molar proportions (mmol/mol) and microbial abundance in*
665 *rumen fluid samples obtained from Charolais-sired (CHX) and purebred Luing (LU) steers*
666 *fed either a high concentrate (Concentrate) or mixed forage:concentrate (Mixed) diets*

Diet (D)	Mixed		Concentrate			Significance		
Breed (B)	CHX	LU	CHX	LU	SEM	B	D	B x D
No of steers	17	19	18	16				
Acetic	645	657	561	577	9.0		***	
Propionic	174	178	293	257	20.7		***	
Butyric	130	118	95	112	17.7		**	†
Valeric	14	14	17	18	0.8		***	
Branched chain[A]	38	34	34	36	10.0			
Copy number (x 10^3) / ng DNA								
Archaea	15.4	11.6	7.4	8.3	3.16		**	
Protozoa	45.8	47.2	34.2	40.5	11.35		†	
Total bacteria	501	565	980	964	69.8		***	
Clostridium								
Cluster IV	156	178	211	289	101.1			
Cluster XIVa	147	174	241	320	87.0		**	
Bacteroides plus *Prevotella*	374	435	994	854	64.4		***	

667 [A]Branched chain: iso-butyric plus isovaleric acids

668 Significance, ***, P<0.001; **, P<0.01; *, P<0.05; †, P<0.1.

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675 **Table 7** *The effect of different metrics on methane emissions from Charolais-sired (CHX)*
676 *and purebred Luing (LU) steers fed either a high concentrate (Concentrate) or mixed*
677 *forage:concentrate (Mixed) diets. Values expressed as a proportion of those for CHX*
678 *steers fed the Mixed diet are given in brackets.*

Diet	Mixed		Concentrate	
Breed	CHX	LU	CHX	LU
Methane				
g / kg DMI	20.2 (1.53)	20.7 (1.57)	13.2 (1.00)	14.7 (1.11)
g/ kg LWG	134 (1.51)	148 (1.66)	90 (1.00)	102 (1.12)
g/kg cold carcass weight	0.567 (1.47)	0.724 (1.88)	0.386 (1.00)	0.525 (1.36)
g/kg total carcass meat	0.794 (1.46)	1.083 (1.99)	0.545 (1.00)	0.762 (1.40)

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682 **Figure Captions**

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684 **Figure 1** Relationships between methane production (g/kg DM intake) and methane to
685 carbon dioxide molar ratio for steers fed Concentrate (solid line and solid circles; CH_4 =
686 7.23 + 124 CH_4 / CO_2 molar ratio, r^2 0.22, P=0.005) and Mixed (broken line and open
687 circles, CH_4 = 10.3 + 141 CH_4/CO_2 molar ratio, r^2 0.10, P=0.060) diets.

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689 **Figure 2** Relationships between methane yield and archaea to bacteria ratio for samples
690 from cattle fed Concentrate (solid line and solid circles CH_4 = 12.5 +160 Archaea to
691 Bacteria ratio, r^2 0.10, P<0.05) and Mixed (open circles, P>0.05) diets.

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